repertoire in the islets is not skewed and the fact that at later stages, the disease becomes lymph node independent.

Nonetheless, it is clear that chemokines do play a role in tissue entry. NOD.*Ccl3*$^{-/-}$ mice show reduced insulitis and are protected from diabetes (Cameron et al., 2000). Furthermore, islet infiltration can be achieved by overexpression of a number of different chemokines driven by the rat insulin promoter even in the B6 background that is not autoimmune prone and that does not normally develop islet infiltration (Luther et al., 2002). It has been suggested that these cells are not islet specific, but it is also conceivable, as would be suggested by the results of Lennon et al. (2009), that they are in fact islet-antigen specific but there are other mechanism of tolerance that prevent islet destruction. The model presented by Lennon et al. will be very useful in addressing the specific role of chemokines in tissue entry of islet-antigen-specific T cells. Additional studies are needed to understand the sequence of events that lead to tissue entry, retention, and destruction.

Perhaps the most important impact of this effort is a renewed opportunity to determine the autoreactive T cells involved in T1D. Attempts to find the antigen responsible for the break in tolerance in diabetes have been complicated by difficulties in cloning antigen-specific T cells. The data present by Lennon et al. suggests that analyzing the infiltrates directly, especially at early stages of disease may be most helpful. Although most of the human studies have been done on patients with clinical symptoms and, therefore, at late stages of disease, and inevitably with peripheral blood as a source of T cells, this work emphasizes the need to look directly at the autoimmune target tissues. Programs such as the JDRF-supported nPOD project (http://www.jdrfnpod.org/) will hopefully provide the tools needed to tackle this problem in humans.

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Gut Immune Balance Is as Easy as S-F-B

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Microbes appear to modulate homeostatic plasticity of T helper and T regulatory cells. In this issue of *Immunity*, Gaboriau-Routhiau et al. (2009) now reveal that segmented filamentous bacteria uniquely coordinate the intestinal T cell profile. The potential implications of this process to various immune functions are discussed.

The human genome encodes information for the development of all of our body's cells, including those of the adaptive immune system. CD4$^+$ T cells serve critical immunologic functions and are primarily involved in mediating resistance to microbial infections. After their lineage commitment in the thymus, naive CD4$^+$ T cells enter the periphery where they receive environmental (extragenomic) signals that further instruct their maturation. During responses to infectious disease, microbial and host signals at the site of infection provide cues to naive T cells to induce their differentiation into various pro- and anti-inflammatory subsets. For example, infection by intracellular pathogens drive the development of T helper 1 (Th1) cells, whereas responses to extracellular pathogens are predominantly of the Th2 and Th17 cell subset (Bettelli et al., 2006). These proinflammatory T helper cells coordinate many aspects of the innate and adaptive immune response to effectively clear microbial invaders. Though teleologic design predicts that the adaptive immune system evolved for this purpose, uncontrolled and indiscriminate T cell responses lead to immunity destructive to the host such as inflammatory bowel disease, autoimmune diseases, and allergies. A primary mechanism to prevent these deleterious reactions is mediated by regulatory T cells (Treg cells) (Sakaguchi et al., 2008). Various subsets of CD4$^+$ Treg cells control

organ-specific autoimmunity and are also induced at the site of infection presumably to dampen immune responses after pathogen clearance. Microbial signals and the immune environment they create during infection modulate the peripheral function of T cells. Furthermore, the proper balance of proinflammatory T helper cells and anti-inflammatory Treg cells critically affects the onset and/or progression of noninfectious immune-mediated diseases.

Recent studies now show that signals from nonpathogenic microbes help "fine-tune" the homeostatic profile of CD4$^+$ T cells (Round and Mazmanian, 2009). In contrast to infections that are relatively rare and opportunistic, mammals are permanently colonized by a diverse collection of commensal microorganisms known as the microbiota. In particular, 100 trillion bacteria of greater than 1,000 species harbored in the mammalian gastrointestinal (GI) tract for the life of the host. By virtue of the sheer magnitude of this interkingdom interaction, it is no surprise that commensal bacteria profoundly affect the immune profile of the host (in the absence of infection). Previous work has shown that germ-free animals (devoid of microbial colonization) display an imbalance in their Th1-Th2 cell profile and have reduced CD4$^+$ T cell proportions (Mazmanian et al., 2005). Colonization with a complex microbiota or even a unique single species of bacteria is sufficient to restore a "normal" immune profile. Therefore, "normal" is defined not by the makeup of the steady-state T helper cell response as instructed by the host genome, but more accurately by the combination of host and microbial genetic instructions. In addition, certain Treg cell populations and Treg cell markers appear to be altered in germ-free animals (Round and Mazmanian, 2009). At least in the intestine, Th17 cell development appears to be exquisitely dependent on microbial colonization. Several recent reports have shown that remarkably, germ-free animals have highly reduced (if not missing) gut Th17 cells (Atarashi et al., 2008; Ivanov et al., 2008). Because Th17 and Treg cell development appear to be highly coordinated, commensal microbes appear to influence the T helper:Treg cell balance through various molecular mediators (e.g., microbial DNA, microbial ATP) (Chow and Mazmanian, 2009). Furthermore, and perhaps most importantly, not all commensal bacteria have a similar ability to induce intestinal T helper cell development. Therefore, the implications from these studies are that defined members of the microbiota have evolved the unique ability to direct specific aspects of immune system maturation.

The report by Gaboriau-Routhiau et al. (2009) in the current issue of *Immunity* now identifies a single microbial species that appears to have the broad ability to coordinate gut T cell responses during homeostatic colonization (Figure 1). Numerous pathogenic microbes are known to elicit Th1, Th2, or Th17 cell responses during infections. This does not appear to be too surprising, with the interpretation being that proper immune recognition of the pathogen results in a synchronization of the suitable T cell response to clear an infection. This notion is further supported by evidence that deletion of a specific arm of the T helper cell response (e.g., T-bet-deficient mice without a Th1 cell response) results in an animal susceptible to the particular category of pathogen the T helper cell response controls (e.g., T-bet-deficient mice are susceptible to the intracellular pathogen *Mycobacterium tuberculosis*) (Sullivan et al., 2005). Bearing in mind the extraordinary diversity and complexity of the intestinal microbiota, how can specific microbes with discrete biological functions be identified? The approach used by Gaboriau-Routhiau et al. (2009) to find the "microbial needle in the haystack" appears as serendipitous as it is rigorous. Initially, transcriptome analysis showed that whereas colonization of germ-free animals with a complex murine microbiota induced gene expression similar to conventionally raised mice, colonization of germ-free mice with human fecal microbiota did not induce the same changes. This is surprising because the human microbiota contains a complex consortium of microbes and suggests a nonredundant or unique role for particular microbial species. Furthermore, when the mouse microbiota was cultured under laboratory conditions and used to colonize germ-free animals, cytokine production in the gut by the culturable constituency appeared similar to germ-free animals, implying the microbe(s) of interest are unculturable. FISH (*F*luorescent *i*n-situ *h*ybridization) analysis revealed that bacteria of the *Clostridium* group were selectively depleted from cultured murine and human bacteria. Because these microbes are heat-resistant spore formers, heating of the donor microbiotas retained the immunomodulatory activity. On the basis of

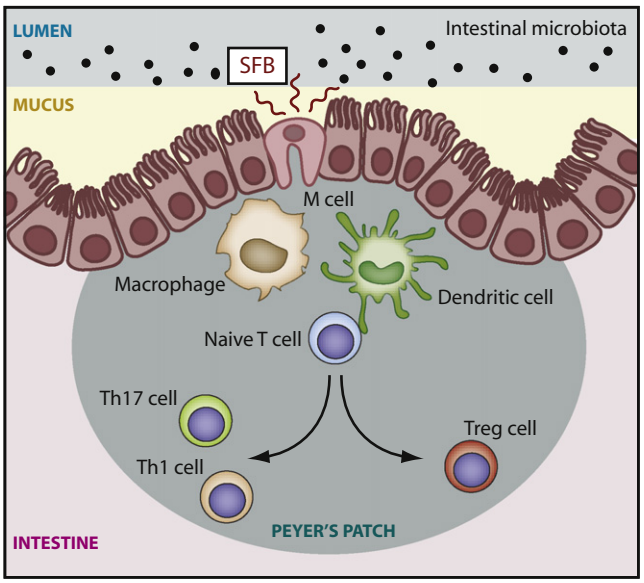

Figure 1. Segmented Filamentous Bacteria Adhere Tightly to the Gut Epithelium and Coordinate T Cell Responses

Most commensal microorganisms reside in the gut lumen, spatially separated from the host immune system. Gaboriau-Routhiau et al. (2009) show that segmented filamentous bacteria (SFB) make close contacts with the mucosal epithelium near Peyer's patches. Colonization with SFBs (but not many other commensal species) induces the expression of a variety of innate and adaptive immune genes in the gut. Furthermore, germ-free animals mono-associated with SFBs develop Th1, Th17, and Treg cell populations similarly to animals colonized with a complex microbiota. It appears SFBs have the unique and nonredundant capacity to influence T helper and T regulatory cell populations in the gut.

clues from the pioneering work of John Cebra who demonstrated that segmented filamentous bacteria (SFBs) have strong immune stimulating properties (mainly analyzing antibody responses) (Talham et al., 1999), the authors hypothesized that SFBs, a spore forming *Clostridium* species, may be the "missing link" as the specific microbe that coordinates homeostatic intestinal T cell responses.

Globally, transcriptional profiling determined by microarray analysis revealed a strong clustering of numerous genes between conventional and SFB mono-colonized mice. Immune pathways including mucosal expression of RegIIIγ, interferon-γ (IFN-γ), interleukin-1β (IL-1β), IL-10, IL-17, iNOS, and IL-12p40 were similar between animals with a complex microbiota and those harboring SFBs alone. It has to be noted that the degree of SFB colonization waned over time, but gratifyingly, so did the amounts of the aforementioned immune transcripts. The lack of SFBs in the culturable microbiota from human and murine donors (which did not induce immune responses) further supported the notion that SFBs possess a unique immunomodulating function, whereas potentially hundreds of other gut bacterial species do not. As shown by scanning electron microscopy, SFBs adhere tightly to Peyer's patches of the small intestine and concomitantly induce local expression of IFN-γ, IL-10, and IL-17. Collectively, the gene expression studies performed by Gaboriau-Routhiau et al. (2009) suggest that SFBs are altering T cell profiles during colonization (their effects on antibody production and B cell activation in germinal centers was shown a decade ago). Finally, the authors directly analyzed cytokine expression by specific T cell subsets in animals colonized with a conventional microbiota or mono-associated with SFBs. Compared to germ-free animals, SFB colonization increased IFN-γ production among $CD4^+$ T cells (Th1), IL-17 production among $CD4^+$ T cells (Th17), and the total number of $CD4^+CD25^+Foxp3^+$ Treg cells among lamina propria lymphocytes of both the small intestine and colon. Notably, in almost all cases, the numbers of T helper and Treg cells in SFB mono-colonized animals did not approach those of mice with a complete microbiota, suggesting that SFBs are sufficient for these changes, but may synergize with other organisms to coordinate the full maturation of intestinal T cell profiles. Taken together, the findings reported by Gaboriau-Routhiau et al. (2009) reveal that a single murine commensal bacterium possesses the unique capacity to induce the development of a multifaceted adaptive immune response in the gut.

Recent studies have demonstrated that the microbiota can have a profound and long-lasting effect on the development of our immune system both inside and outside the intestine. Gaboriau-Routhiau et al. (2009) show that a single bacterium, SFBs, profoundly alter the profile of both pro- and anti-inflammatory T cells in the gut. Numerous unsolved mysteries about this process will make the forthcoming investigations in this area very exciting. For example, what bacterial molecule(s) mediate the SFB effect? How can a single organism coordinate such a diverse immunological response; i.e., what cells of the immune system (epithelial cells, dendritic cells, macrophages, etc.) sense and respond to SFBs? Perhaps, more importantly, it remains to be seen what effects (if any) this process has on host health. Proinflammatory T helper cells are critical in controlling microbial infections; are SFB-colonized animals better able to fight pathogens than animals without SFBs? Furthermore, aberrant Th1, Th2, and Th17 cell reactions lead to host pathologies (autoimmunity and allergies), if not controlled by Treg cells (Bettelli et al., 2006). Potentially, the dialog between the host and microbe can influence the development of diseases such as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, type 1 diabetes, and asthma. Mounting evidence suggests the hypothesis that alterations in the gastrointestinal microbiota (due to recent lifestyle changes) have disrupted microbial-mediated mechanisms of immunological tolerance within and outside the gut (Round and Mazmanian, 2009). In other words, the composition of the microbiota can affect various immunologic diseases in humans. It appears that the convergence between the fields of microbiology and immunology will reveal novel biological paradigms about our intimate association with the microbial world and how these associations affect our health.

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